

Evaluation of micronuclei in tobacco users: A study in Punjabi population

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Abstract

Introduction: The assessment of micronuclei in exfoliated cells is a promising tool for the study of epithelial carcinogens and can be used to detect chromosome breakage or mitotic interference, thought to be relevant to carcinogenesis. **Aim:** The present study aimed to detect micronuclei in exfoliated oral mucosal cells in individuals using various tobacco forms from the last 5 years. **Materials and Methods:** A total of 75 healthy male subjects (25 smokeless tobacco users, 25 smokers, and 25 non-tobacco users) were selected for the study. Smears were obtained with moistened wooden spatula from buccal mucosa and fixed with 95% alcohol. All the cytologic smears were stained by Papanicolaou technique. From each slide, ~1000 cells were examined under the 400× magnification and where micronucleated (MN) cells were located, they were examined under the 1000× magnification. **Result:** MN cells were found to be significantly higher in smokeless tobacco users than in smokers and controls. **Conclusion:** A positive correlation is found between increased micronucleus frequency and tobacco-using habits. So micronucleus assay can be used as a biomarker of genotoxicity.

Keywords: Chromosomal aberrations, exfoliative cytology, genotoxic agents, micronuclei, Papanicolaou stain

Introduction

Cancer is one of the most common causes of morbidity and mortality today. The global burden of cancer continues to increase mostly because of increasing adoption of cancer-causing behaviors, particularly smoking and smokeless tobacco forms in economically developing countries. Globally, about 5,00,000 new oral and pharyngeal cancers are diagnosed annually and three quarters of these are seen in the developing world, including about 65,000 cases reported in India. The World Health Organization (WHO) estimated that the proportion of deaths that result from tobacco-related diseases will rise in India from 1.4% of all deaths in 1990 to 13.3% of all deaths in 2020. The number of persons consuming tobacco is also likely to rise, according to the models presented in the 2002 report of the Economic and Social Council (ECOSOC) of the United Nations.^[1,2]

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Oral carcinomas are characterized by complex karyotypes that involve many chromosomal deletions, translocations, and structural abnormalities. Cells often have errors in chromosome segregation that lead to the formation of a lagging chromosome or chromosome parts that become lost during the anaphase stage of cell separation and are excluded from the reforming nuclei. The laggards are observed in the cytoplasm as micronuclei.^[3]

The micronuclei are extranuclear cytoplasmic bodies associated with chromosomal aberrations. These are induced in oral exfoliated cells by a variety of substances, including genotoxic agents and carcinogenic compounds in tobacco, betel nut, and alcohol. The induction of micronucleated (MN) cells by carcinogens and mutagens is a sign of the genotoxic effect of such substances.^[4] These are also observed in exfoliated buccal cells, from people who are exposed to organic solvents, antineoplastic agents, diesel derivatives, polycyclic aromatic hydrocarbons, lead-containing paints and solvents, and drinking water, which is contaminated with arsenic.^[3]

The assessment of micronuclei in exfoliated cells is a promising tool for the study of epithelial carcinogens and can be used to detect chromosome breakage or mitotic interference, thought to be relevant to carcinogenesis.^[5] Due to its association with chromosomal aberrations, micronuclei have been used since 1937 as an indicator of genotoxic exposure based on the radiation studies conducted by Brenneke and Mather.^[6] The direct correlation between the micronuclei formation and genomic damage make the micronuclei assay an efficient alteration to the metaphase analysis.^[4]

Aim

The present study aimed to detect micronuclei in exfoliated buccal mucosal cells in healthy tobacco users and healthy nontobacco users.

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Materials and Methods

A total of 75 healthy male subjects (25 smokeless tobacco users, 25 smokers, and 25 non-tobacco users) who attended the outpatient department of Dental Institute, were examined and were selected for the study. The study group comprised smokeless tobacco chewers who chewed five or more packets daily for at least 5 years. The smokers included smoked every day for at least 5 years and consumed 20 or more bidis/cigarettes in a day. The control group persons were not habituated to any form of tobacco consumption. Individuals who were alcoholics, had a recent viral infection, had undergone radiation therapy, or those who had been under medication were excluded from the study.

Cytologic preparation

Before sampling, each individual rinsed his mouth thoroughly with tap water. The exfoliated cells were obtained by scraping the buccal mucosa with a moistened wooden spatula and the scraped cells were placed on the clean glass slides and smears were prepared. The smears were fixed with 95% ethyl alcohol. All the cytologic smears were stained by Papanicolaou technique using a commercially available staining kit RAPIDPAP. From each slide, ~ 1000 cells were examined under the $\times 400$ magnification and where MN cells were located, they were examined under the $1000\times$ magnification. The criterion which was developed by Tolbert *et al* was used for counting the micronuclei.

Tolbert *et al* criteria^[5] parameters for identifying micronucleus are as follows:

1. Rounded smooth perimeter suggestive of a membrane.
2. Less than a third the diameter of associated nucleus, but large enough to discern shape and color.
3. Staining intensity similar to nucleus.
4. Texture similar to nucleus.
5. Same focal plane as nucleus.
6. Absence of overlap with or bridge to nucleus.

Dead or degenerating cells (karyolysis, karyorrhexis, nuclear fragmentation) were excluded from evaluation. Nuclear blebblings (micronucleus-like structure connected with the main nucleus with a bridge) were also not considered.

Results and Analysis

The MN cells observed are shown in Figures 1–3.

The frequency of distribution of micronuclei in the three study groups are shown in Figures 4–5.

The mean number of micronuclei observed in the three groups are presented in table 1. The mean number of micronuclei in smokeless tobacco chewers, smokers, and controls were 24.13 ± 10.68 , 11.96 ± 4.23 , and 4.17 ± 2.99 , respectively. The mean number of micronuclei found

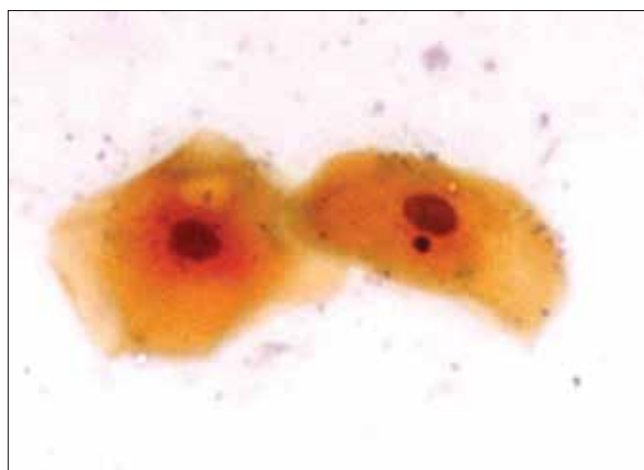


Figure 1: Cell with a micronucleus Pap ($\times 1000$)

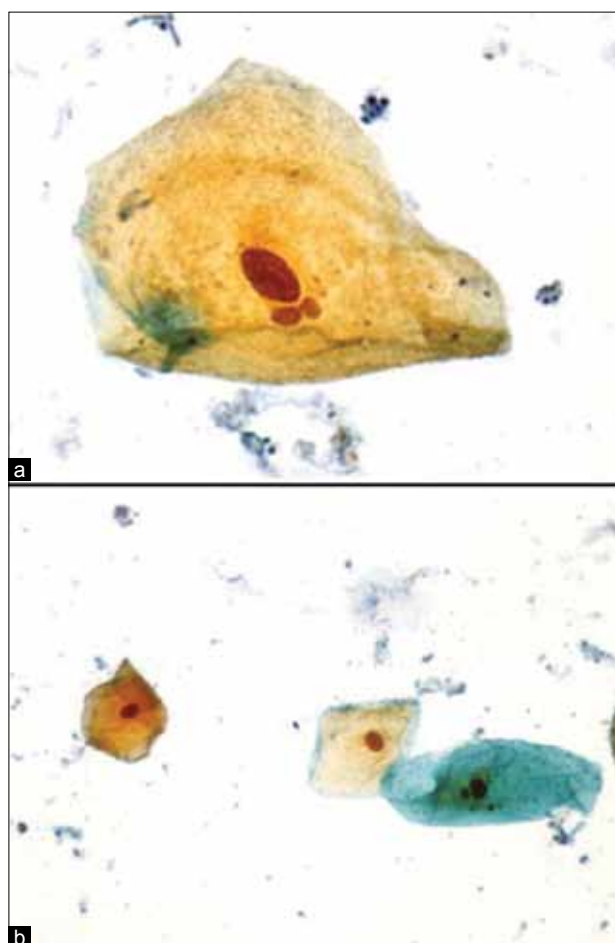


Figure 2: (a) Cell with two micronuclei Pap ($\times 1000$); (b) cell with two micronuclei Pap ($\times 1000$)

were more in smokeless tobacco chewers as compared with smokers and controls [Table 1].

The mean values and the mean differences, which were obtained from the smokeless tobacco chewers, the smokers, and the controls were compared and are shown in table 2.

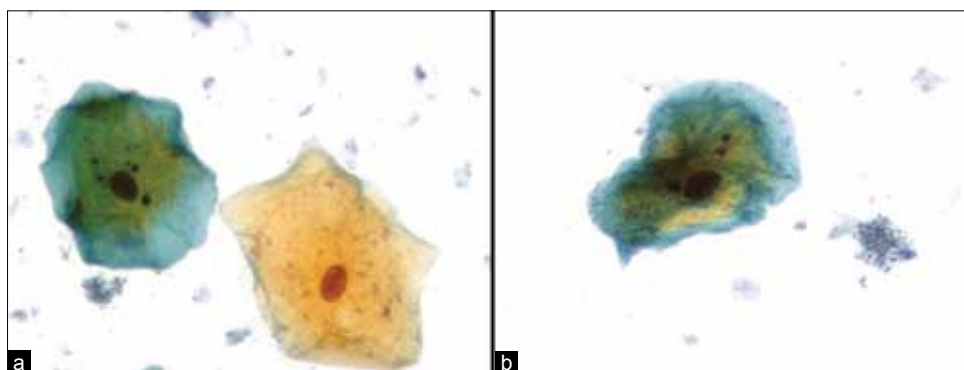


Figure 3: (a) Cell with three micronuclei Pap (x1000); (b) cell with three micronuclei Pap (x1000)

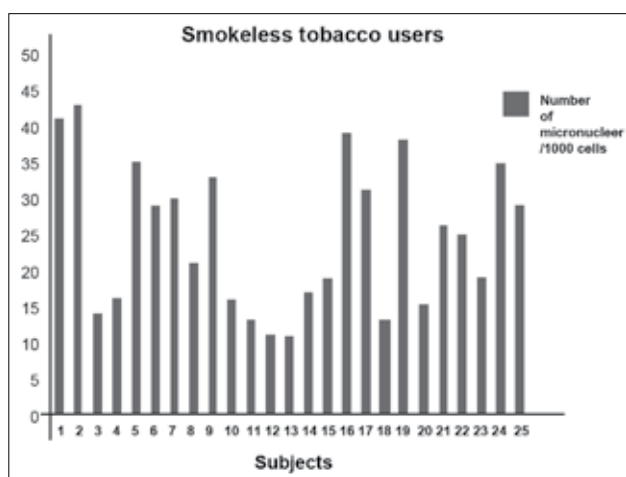


Figure 4: Number of micronuclei/1000 cells in smokeless tobacco users

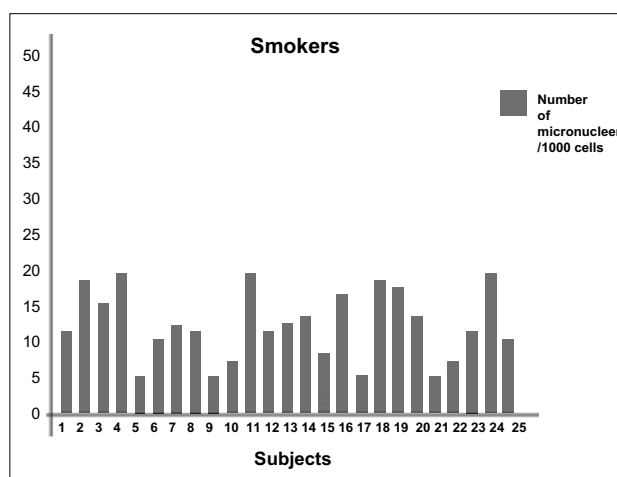


Figure 5: Number of micronuclei/1000 cells in smokers

Table 1: Mean number of micronuclei in three study groups

Groups	N	Mean	Standard deviation
Smokeless tobacco users	25	24.13	10.68
Smokers	25	11.96	4.23
Control	25	4.17	2.99

In comparison, the mean difference between the number of micronuclei in smokeless tobacco chewers and smokers was 12.17 and was statistically significant ($P < 0.05$). The mean difference between the number of micronuclei in smokeless tobacco chewers and controls was 19.96 and was highly statistically significant ($P < 0.05$). The mean difference between the number of micronuclei in smokers and controls was 7.79 and was statistically significant ($P < 0.05$) [Table 2].

Discussion

Oral carcinogenesis is a multistep process of accumulated genetic damage leading to cell dysregulation with disruption in cell signaling, DNA-repair, and cell cycle events, which are fundamental to hemostasis. These events can be conveniently studied in the buccal mucosa, which is an easily accessible

Table 2: Multiple comparison of micronuclei in three study groups

Groups	N	Mean \pm Standard deviation	Mean difference
Smokeless tobacco users	25	24.13 \pm 10.68	12.17
Smokers	25	11.96 \pm 4.23	
<i>P</i> value		0.00487	
Smokeless tobacco users	25	24.13 \pm 10.68	19.96
Control	25	4.17 \pm 2.99	
<i>P</i> value		0.00286	
Smokers	25	11.96 \pm 4.23	7.79
Control	25	4.17 \pm 2.99	
<i>P</i> value		0.00343	

tissue for sampling cells in a minimally invasive manner and does not cause undue stress to study subjects.^[5]

Oral exfoliative cytology has been used extensively for screening cellular alterations, such as karyolysis, karyorrhexis, micronucleus formation, pyknosis, binucleation, broken egg nucleus, anucleation, and so on^[5]. Micronuclei in oral exfoliated cells is a biomarker of chromosomal damage

caused by genotoxic agents from tobacco and tobacco-related substances, alcohol, and so on. When the target tissue of interest is epithelial tissue, the exfoliated cell micronucleus assay has been used to assess the genotoxic damage in oral premalignancies and oral squamous cell carcinoma.^[7,8]

The present study evaluated the mean number of micronuclei in smokeless tobacco users, smokers, and the healthy control group. The result showed that the overall level of mean number of micronuclei in smokeless tobacco were higher (24.13 ± 10.68) as compared with smokers (11.96 ± 4.23) and controls (4.17 ± 2.99). This observation was similar to those reported by Palaskar *et al.*,^[3] Ozkul *et al.*,^[9] and Patel *et al.*,^[10] when all the groups were further compared with each other for the mean difference, the result was highly statistically significant ($P < 0.05$), which was in accordance with the previous studies by Palaskar *et al.*^[3] and Patel *et al.*,^[10] whereas Ozkul *et al* found no difference between the mean percentage of MN cells for the groups considered ($P > 0.05$).^[9]

The incidence of micronuclei has been analyzed by various studies in normal patients, oral premalignancies, and OSCCs.^[7] Casartelli *et al* observed micronuclei frequencies in exfoliated buccal cells in normal oral mucosa, precancerous lesions, and squamous cell carcinoma. They concluded that the gradual increase in micronucleus counts from normal mucosal to precancerous lesions to carcinoma suggested a link of this biomarker with neoplastic progression.^[11] A significant rise was also observed in the percentage of MN cells and micronuclei from control to precancer patients, and from precancer to cancer patients in a study by Saran *et al.*^[12] In the present study, the mean number of micronuclei was higher in smokeless tobacco users indicating more genotoxic effect of smokeless tobacco as compared with smoking.

Conclusion

From the present study, increase in the number of micronuclei provides the evidence that smokeless tobacco chewers and smokers may be at a high risk for developing oral cancer. In comparison, the cellular changes associated with smokeless tobacco use were more than that in smokers, thus indicating more carcinogenic potential of smokeless

tobacco. Micronucleus assay can be used as a biomarker of genotoxicity and epithelial carcinogenic progression. However, more research is required to establish it as a potential biomarker for oral carcinogenesis.

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